Journal of the American Mosquito Control Association, 13(4):297–304, 1997

FIELD TRIALS WITH *BACILLUS SPHAERICUS* FORMULATIONS AGAINST POLLUTED WATER MOSQUITOES IN A SUBURBAN AREA OF BANGKOK, THAILAND

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ABSTRACT. Two newly developed *Bacillus sphaericus* larvicidal formulations, VectoLex CG® (corncob granules) and VectoLex WDG® (water dispersible granules), were tested against *Culex quinquefasciatus* larvae in 4 highly polluted breeding sites in Thailand. VectoLex CG, applied at rates of 0.5–2 g/m^2, gave satisfactory to complete control of late-instar larvae and pupae for up to 4 wk after treatment. The VectoLex WDG, which had higher potency and was applied at rates of 0.1–0.5 g/m^2, gave satisfactory control for 1–4 wk after treatment. Among the factors influencing longevity of control were dosage of a given formulation, precipitation, and flooding of the treated sites; the latter had the greatest impact. Presence of larvivorous fish did not seem to influence larval populations because there were heavy populations of mosquito larvae present in the test sites in the presence of moderate numbers of fish before the application of *B. sphaericus* treatments.

INTRODUCTION

Bacillus sphaericus Neide is a spore-forming bacterium found commonly in soil, water, and other substrates in nature. Most strains of this bacterium are nonpathogenic to insects. However, in recent years, a number of strains have been discovered and isolated that produce parasporal toxins that are toxic to larvae of several genera of mosquitoes. The binary crystalline toxins produced by *B. sphaericus* strain 2362 have high activity against larvae of *Culex* mosquitoes (Mulla 1991). A corncob granular formulation of this strain with the trade name of VectoLex CG® (Abbott Laboratories, North Chicago, IL) was registered for mosquito control by the United States Environmental Protection Agency in 1991. This formulation and others are just now becoming available for experimental use and for the control of mosquitoes around the world.

Bacillus sphaericus formulations have been tested and evaluated in a variety of habitats, especially the breeding sites of *Culex* mosquitoes. Karch et al. (1991) evaluated a granular formulation (VectoLex) of this microbial larvicide against *Anopheles gambiae* Giles in clear water and *Culex quinquefasciatus* Say in polluted water in Zaire. The granular formulation was found to be highly effective against the polluted water mosquito. Similarly, *B. sphaericus* formulations were found to show good activity against *Anopheles stephensi* Liston (Kumar et al. 1994).

The most important and desirable attributes of a microbial larvicide formulation are persistence and recycling, as well as suspension of the particulate toxins in the feeding zone of mosquito larvae. Unfortunately, most evidence to date points out that the toxin particles in currently available formulations settle out from the feeding zone of larvae and sink to the bottom of the water (Davidson et al. 1984, Mulla et al. 1988, Matanmi et al. 1990). Recycling of *B. sphaericus* at least under laboratory conditions has been suggested by numerous workers. Mosquito larval cadavers have been reported to serve as substrates for spore production (Becker et al. 1995, Correa and Yousten 1995). Nicolas et al. (1987) reported persistence and recycling of *B. sphaericus* in a field test in West Africa. The studies of Skovmand and Bauduin (1997) clearly showed the recycling of this agent where larvae were added to treated regimens, the dead larvae acting as a medium for the production of spores.

Because field studies on the persistence and longevity of larval control with *B. sphaericus* formulations, especially in polluted waters, are few, the present studies were initiated to determine the efficacy of 2 newly developed formulations of *B. sphaericus* against *Culex* larvae in highly polluted water habitats in the Nonthaburi Province in the suburb of Bangkok. To find the minimum effective dosage, various rates of the experimental formulations were evaluated for initial efficacy as well as long-term control of mosquito larvae. The studies were initiated in August 1996 and continued into January 1997.

MATERIALS AND METHODS

Four small breeding sites, of various surface areas, where heavy populations of *Culex* larvae prevailed were selected for these studies. Mosquito larvae breeding in these habitats were primarily *Cx. quinquefasciatus.* The formulations evaluated and the site descriptions are as follows.

Formulations

VectoLex CG corncob granules with 50 ITU/mg (lot no. 16-723-NB containing 7.5% of a technical powder of *B. sphaericus*) and VectoLex WDG® wa-

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ter dispersible granules with 350 ITU/mg (designated as ABG-6461, lot no. 21-105-BR) were provided by Abbott Laboratories. It should be noted that the potency and characteristics of the VectoLex corncob granules have changed over time, and the new formulation evaluated here was different from those studied earlier. The VectoLex WDG formulation tested here was also a new product that has not been field tested previously.

Sites

The four mosquito breeding sites used in these experiments were located in a low income area of Nonthaburi Province in the suburbs of Bangkok, Thailand. All sites received wastewater from dwellings that were raised on posts or pillars. Wastewater accumulated in depressions both under the houses and in open areas around the houses. There was a great amount of solid waste in the water as well as on the ground above the water line. Plastic containers and bags as well as plastic and glass bottles and decaying plant wastes constituted the bulk of the solid waste mass. Solid waste accumulation created discontinuous water pools, some of them very small and holding a few hundred ml of water. The accumulated water came from washing clothes, kitchen utensils, and household wares, from bathing and showers, and, in some cases, from leaky or broken sewers and septic tanks. Frequent rains during the early part of the experimental period also added water. At times, there was substantial precipitation that flooded the catwalks, the only access to the communities and dwellings from paved city streets. Because of the continuous addition of domestic wastewater and rainwater, there was some water flow into and out of the treated plots, but in the absence of rain, the flow rate was minimal.

Thanausi community: This site, which consisted of a ditch and pool extended under and around a house, received wastewater from domestic sources. The area treated with VectoLex CG and WDG granules was about 50 m^2. This site was treated 4 times, the first 2 treatments with VectoLex CG, and the last 2 with the WDG formulation. VectoLex CG was applied at the rate of 2 g/m^2 on September 3 and October 17, 1996. The WDG applications were made on November 14 and December 11, 1996 at the rate of 0.25 g/m^2, a lower dosage than that of VectoLex CG because the WDG formulation possessed high potency.

Raevadee community: This site consisted of a pool and depression under and around houses that received wastewater from domestic uses. The area treated with VectoLex CG and WDG granules was about 100 m^2. The VectoLex CG formulation was used at the rate of 2 g/m^2 in the first 2 treatments made on September 3 and October 17, 1996. This same area was then treated twice with the WDG formulation at the rate of 0.1 g/m^2 and 0.25 g/m^2 (lower dosages than that of VectoLex CG because of the high potency of the WDG) on November 14 and December 25, 1996, respectively.

Wat Lannaboon community: This site consisted of a small ditch that opened into a larger area under and beside houses. Again, wastewater from domestic uses accumulated in this area. The total area of the plot initially treated (only the ditch) was 50 m^2; it was treated twice at the rate of 0.5 g/m^2 of VectoLex CG on September 5 and October 17, 1996. For the WDG treatments, the area was increased to 100 m^2 and treated on November 14 at the rate of 0.25 g/m^2 and again on January 4, 1997 at the rate of 0.5 g/m^2 of the WDG formulation.

Wat Tinnakornnimit: This site consisted of a small pool of highly polluted water under and beside houses that received wastewater from the dwellings and also contained a large amount of solid waste and floating debris. An area of about 16 m^2 was administered 2 treatments of VectoLex CG at the rate of 2 g/m^2 on September 3 and 20, 1996 and a 3rd treatment of 1.6 g/m^2 on October 17, 1996. The WDG formulation was applied twice (November 14 and December 11, 1996) at 0.1 g/m^2.

Applications

The required amounts of the VectoLex CG corncob granules were broadcast by hand as evenly as possible. With this method of application, it was not possible to treat water under the houses; only the open accessible areas were covered. For assessment of larval populations before and after treatment, only the accessible areas were sampled.

The WDG granules were easily suspended in a small amount of water in a hand-operated compression spray tank. The tank was filled with 2–5 l of water (depending on area of the plot) and manually shaken. This action was sufficient to completely suspend the formulation in water with no need for further agitation. The tank was pressurized and the water suspension sprayed out through a T-jet nozzle producing coarse spray. The spray stream could be directed under the houses and could reach 1–2 m from the edge inward.

Sampling

Larval populations were sampled in all sites by the standard dipping technique using a 400-ml dipper and taking 5–10 dip samples at each site and at each interval depending on plot size. Samples were taken in a biased manner, sampling those spots where mosquito larvae were noted in large numbers. For counting, the contents of the dipper were transferred to white plastic trays (15 × 30 × 4 cm deep), and the larvae were counted and categorized as 1st and 2nd instars and 3rd and 4th instars and pupae. The sites were sampled before and at intervals (as shown in the figures) after treatment. When the counts of 3rd and 4th instars and

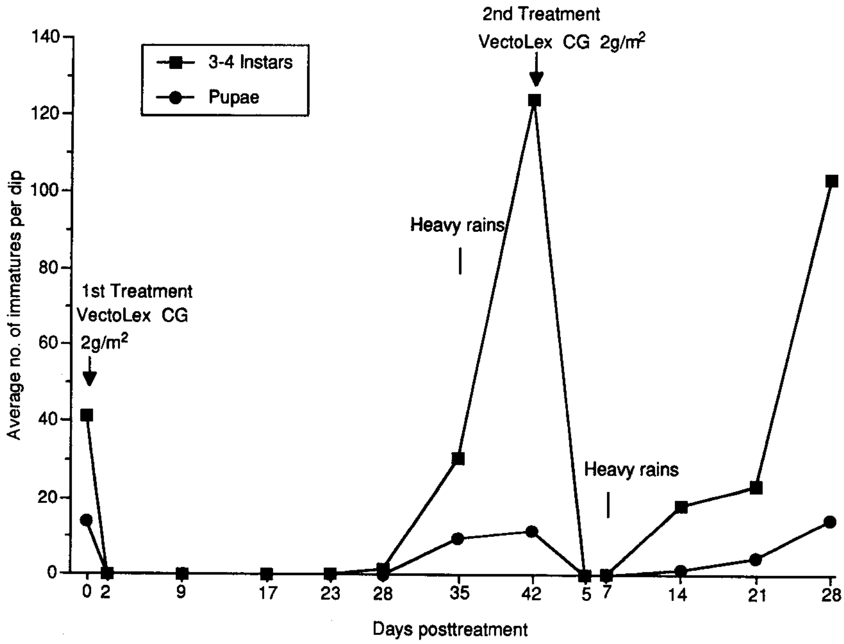

Fig. 1. Evaluation of VectoLex CG® of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Thanausi community, Nonthaburi, Bangkok (1996).

pupae resurged, reaching or exceeding the pretreatment counts, the sites were retreated with the same or different dosage or a different formulation. The numbers of 1st and 2nd instars, although counted, are omitted from the figures because they are not a good indicator of the level of control. *Bacillus sphaericus* acts slowly on mosquito larvae, and 1st and 2nd instars can survive for 24–48 h before they suffer mortality. This is especially true of 1st instars, which could have hatched a few hours before assessment and would not have had a sufficient exposure period.

RESULTS

Thanausi community: The 1st treatment at the rate of 2 g/m² of VectoLex CG formulation produced complete control of late-instar larvae and pupae for almost 28 days (Fig. 1). Five weeks after treatment, there was reappearance of 3rd- and 4th-instar larvae, as well as pupae. This resurgence was preceded by rains, which possibly flushed the treated site. The populations further increased, and the site was then retreated with VectoLex CG at the same rate.

The 2nd treatment of VectoLex CG produced complete control of all immatures for 7 days. On the 7th day, heavy rains occurred, and the flushing out of the treated site resulted in slight resurgence of larvae 14 and 21 days after treatment, but the pupal population was still low (Fig. 1). One month after this 2nd treatment, the larval and pupal populations increased markedly, reaching or exceeding the pretreatment populations, at which time the site was treated with the WDG formulation.

The 3rd and 4th treatments were made with the VectoLex WDG formulation at 0.25 g/m² (Fig. 2). After the 3rd treatment, the immatures almost reached zero 4 and 7 days posttreatment, but the numbers of immatures increased slightly 14 days posttreatment. Population recovery, especially 3rd and 4th instars, was substantial 21 and 28 days posttreatment, after which time the site was retreated with the 4th application of WDG. This treatment markedly suppressed all immatures for 14 days, after which time there was some recovery of larvae. Recovery was complete in 1 month, but the populations declined 6 wk posttreatment. At this site, a good number of larvivorous fish (either *Gambusia* or *Poecilia,* species not determined) prevailed in the open water. Even with fish present, there were heavy populations of mosquito larvae prevailing in protected areas.

Raevadee community: This site supported heavy populations of immature mosquitoes prior to the 1st treatment. VectoLex CG treatment at the rate of 2 g/m² suppressed the larval populations markedly, and the pupal population reached zero 2 days after treatment (Fig. 3). Nine days posttreatment, the larval populations increased slightly but were still considered low for this site, and the pupal population remained negligible. By 15 days posttreatment, the larval and pupal populations increased substantially. This increase could have been a result of heavy rains (on the 9th day posttreatment) flushing out the site. From 15 days to 6 wk posttreatment, populations of

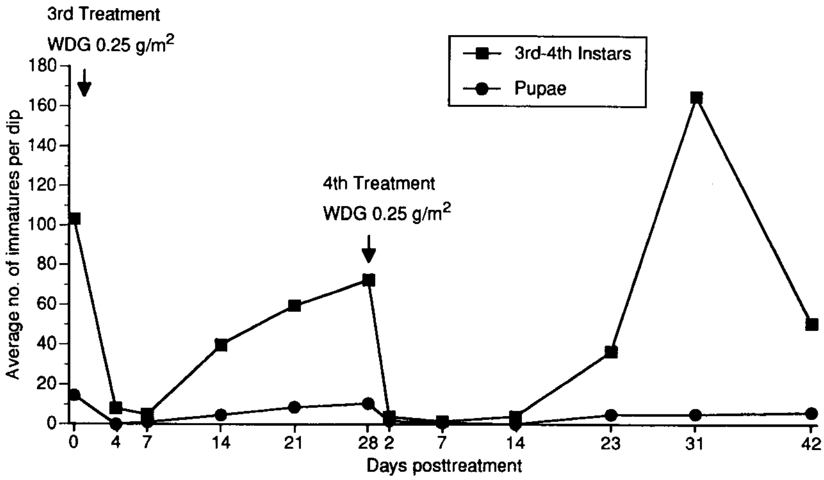

Fig. 2. Evaluation of WDG formulation of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Thanausi community, Nonthaburi, Bangkok (1996).

all immature stages, including the pupae, prevailed in high numbers. At 6 wk posttreatment, the site was retreated at the same rate of VectoLex CG.

The 2nd treatment with VectoLex CG produced complete control of immatures for 7 days posttreatment. Soon after assessment, heavy rains occurred in the area. Because of flooding and flushing of the treated site, there was slight recovery of all immature stages 15 days posttreatment, but the density of immatures was considered low for 15 and 21 days posttreatment. Thirty days after this treatment, populations of immatures increased further. This area as well as additional contiguous area were treated with the WDG formulation at 0.1 g/m². This 3rd treatment with WDG yielded excellent control of the immatures up to 28 days posttreatment (Fig. 4). Because of the lack of precipitation, even this low dosage of WDG yielded excellent control of larvae and pupae for 28 days. There was a marked recovery of the immatures (especially 3rd and 4th instars) 33 and 40 days posttreatment, when the site was administered the 4th treatment using the WDG formulation at 0.25 g/m². This treatment produced a high level of control 7 days posttreatment. Recovery of larval and pupal populations ensued 15 days posttreatment because of the water flow from an adjacent canal with possible movement of larvae and pupae into the plot and dilution of the *B. sphaericus* toxins.

This site also supported populations of larvivorous fish that were active in the deeper, open parts of the habitat. Despite their presence, this site harbored heavy populations of larvae prior to treatment with *B. sphaericus*.

Wat Lannaboon community: This small site, which consisted of a ditch that flowed continuously, received water from upstream from a pool that accumulated water under and beside a house and could not be treated. The 1st treatment with VectoLex CG at 0.5 g/m² yielded almost complete control of all immature stages 4 days after treatment (Fig. 5). Seven days after treatment, there was slight recovery in the larvae, but the pupal population remained zero. At 13 days posttreatment, there was substantial recovery of all immature stages, but they declined markedly 21 and 28 days posttreatment. At 5 wk posttreatment, there was heavy rainfall and the population recovered, and after 6 wk, the site was retreated with the same rate of VectoLex CG formulation.

The 2nd treatment at 0.5 g/m² of VectoLex CG produced almost complete control of all immatures for up to 7 days, after which heavy rains occurred (Fig. 5). No samples were taken at 14 days posttreatment because flooding made the area inaccessible. The sampling at 21 days posttreatment still showed low populations of larvae and pupae. The last samples at 30 days posttreatment showed good recovery of populations, which equaled the pretreatment level. This area as well as additional water accumulations were then treated with sprays using the WDG formulation.

The 3rd treatment using the WDG formulation at 0.25 g/m² provided good control for 4 days posttreatment, but mediocre control was noted 7 days posttreatment because the site was heavily flooded by rains (Fig. 6). The populations increased further at 14 and 21 days posttreatment, and the site was retreated with WDG at 0.5 g/m². This treatment yielded almost complete control of immatures for 37 days posttreatment. Immature populations remained very low for up to 48 days posttreatment because there was little or no rain during this period at this site.

This breeding source had a low level of larvivorous fish populations. The fish were seen to swim in the open parts of the habitat, leaving the larvae to occupy protected and discontinuous niches.

Wat Tinnakornnimit community: This area con-

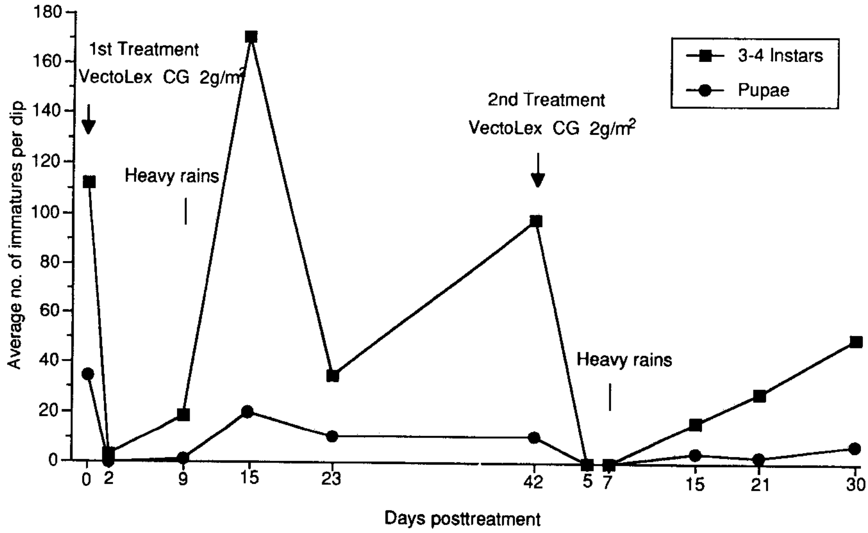

Fig. 3. Evaluation of VectoLex CG of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Raevadee community, Nonthaburi, Bangkok (1996).

sisted of a small pool of highly polluted wastewater accumulated under houses. Prior to treatment with VectoLex CG (2 g/m^2), the pool supported heavy populations of mosquito larvae and pupae. Up to 1 wk posttreatment, all immatures were reduced to very low levels. Slight resurgence occurred 13 and 15 days after treatment, and the site was retreated with VectoLex CG at the same rate (Fig. 7).

The second treatment suppressed immatures markedly although not completely for about 6 days (Fig. 7). There was some recovery 2 and 3 wk posttreatment but notable recovery, of the immatures especially, after the rains occurred 4 wk posttreatment, at which time the site was administered a 3rd treatment at 1.6 g/m^2 of VectoLex CG, which produced almost complete control of immatures for up to 7 days and a high level of control of pupae for up to 29 days (Fig. 7). Twenty-nine days after treatment, there was slight recovery of the larvae, and the area was sprayed with WDG formulation.

The 4th treatment with WDG formulation (0.1 g/m^2) suppressed larval populations for 7 days (Fig. 8). Thereafter, the larval populations increased, and the 5th treatment at the same rate of WDG was

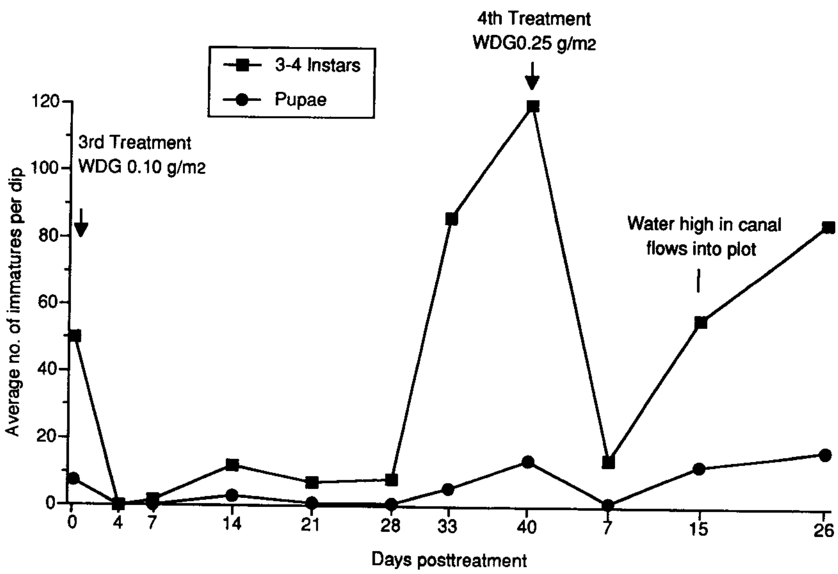

Fig. 4. Evaluation of WDG formulation of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Raevadee community, Nonthaburi, Bangkok (1996).

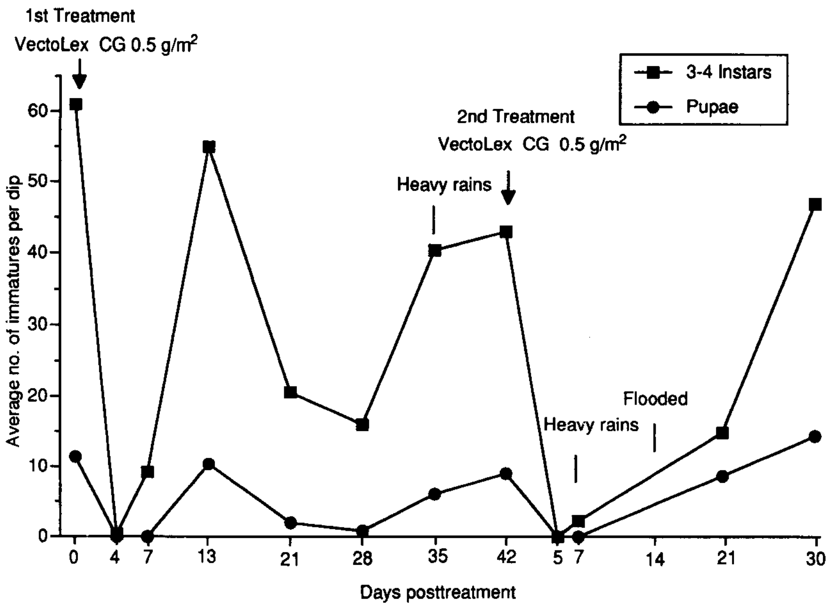

Fig. 5. Evaluation of VectoLex CG of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Wat Lannaboon community, Nonthaburi, Bangkok (1996).

made 28 days after the 4th treatment. This 5th treatment suppressed late-instar larvae significantly, but the pupal population was suppressed markedly for 15 days, after which period all immatures increased in numbers.

This very small treated site was adjacent to larger water accumulations under houses that could be treated 2 m inward at the most under the houses. Encroachment of immatures from the adjacent untreated area and flooding of the small treated area by rains up to the end of the 3rd treatment are possible reasons for lower efficacy of the treatments at this site.

DISCUSSION

It is evident from these field experiments that *B. sphaericus* formulations can provide excellent control of mosquitoes in polluted waters. The persistence and longevity of efficacy to a large extent depended on formulations, rate of application, and

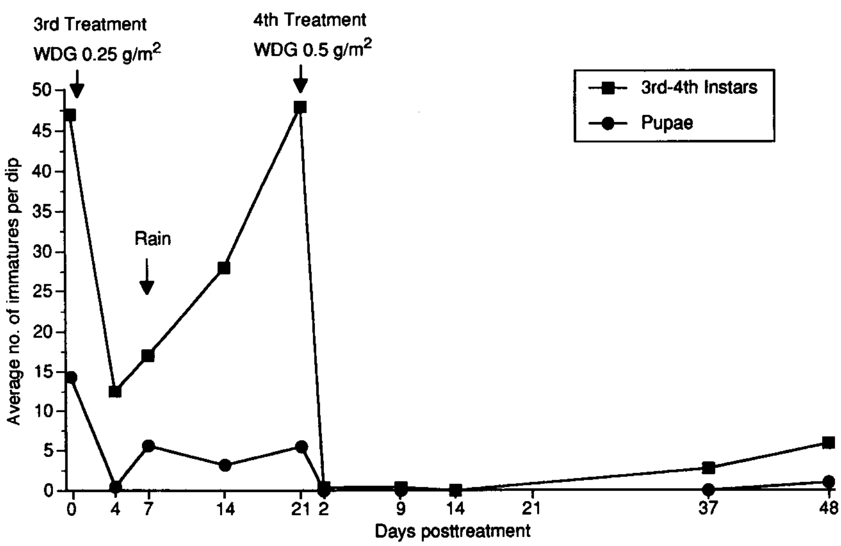

Fig. 6. Evaluation of WDG granular formulation of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Wat Lannaboon community, Nonthaburi, Bangkok (1996).

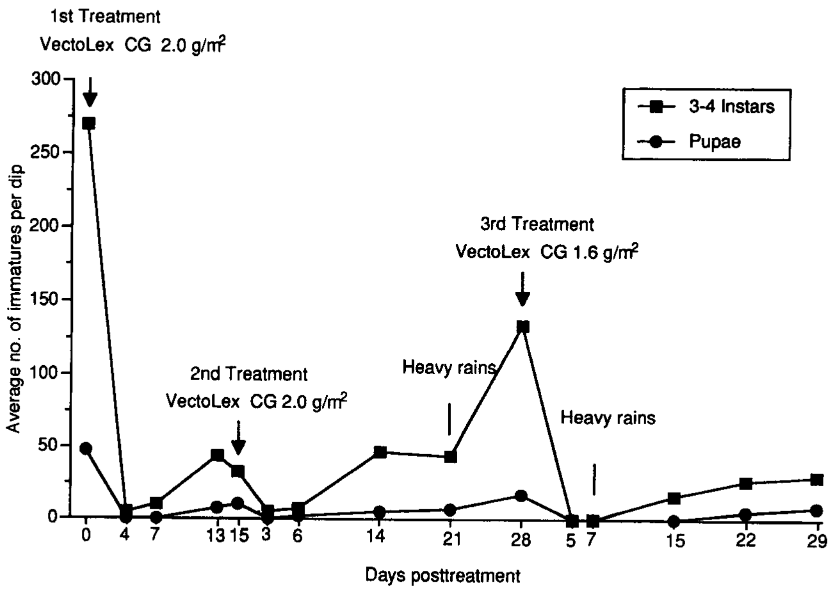

Fig. 7. Evaluation of VectoLex CG of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Wat Tinnakornnimit community, Nonthaburi, Bangkok (1996).

extent of rains and flooding. Although some of the sites supported low populations of larvivorous fish, the role of the fish in control of larvae seemed to be minor. Even with fish present, these sites supported heavy populations of mosquito larvae prior to treatment. The 1st treatment with VectoLex CG at the rate of 2 g/m² of the formulation provided excellent suppression of mosquito larvae and pupae for 28 days at Thanausi, after which period there was a significant population resurgence following heavy rains. The 2nd treatment at the same rate at this site provided good control for only 14 days because of heavy rains (7 days posttreatment) flushing out the site (Fig. 1). Similarly, the 1st treatment of VectoLex CG (2 g/m²) at Raevadee provided excellent control but for only 9 days. On the 9th day, the site was flooded by heavy rains, and the population fully recovered on the 15th day (Fig. 3). The 2nd treatment of VectoLex CG at the same rate provided control for 15 days because heavy rains 7 days posttreatment again flooded this site (Fig. 3). Another treatment with VectoLex CG (2 g/m²) of a very small site (Wat Tinnakornimit) yielded excellent control for 13 days; the 2nd treatment (the same rate) at this site produced excellent control for 3 wk, after which heavy rains caused decline in

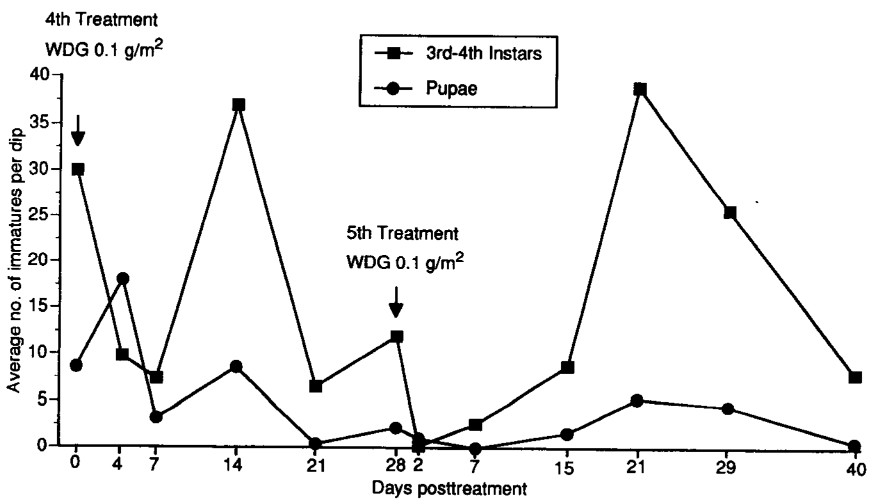

Fig. 8. Evaluation of WDG formulation of *Bacillus sphaericus* (strain 2362) against larvae of *Culex quinquefasciatus* in domestic polluted waters in Wat Tinnakornnimit community, Nonthaburi, Bangkok (1996).

efficacy. A 3rd treatment with VectoLex CG (1.6 g/m^2) yielded excellent control for 15 days, after which the efficacy declined somewhat as a result of rains 7 days posttreatment (Fig. 7) but still kept the immature population at a low level.

At one site (Wat Lannaboon) receiving a low-dosage treatment of VectoLex CG (at 0.5 g/m^2), the efficacy of this treatment lasted for only 7 days despite the fact that there was no heavy rain (Fig. 5). The 2nd treatment at the same rate also yielded a high level of control for 7 days, and the efficacy declined when the area was flooded and could not be sampled 14 days posttreatment (Fig. 5). This treated area was very small and was surrounded by larger mosquito breeding areas that could not be treated. It is likely that larvae and pupae moved into the treated plot.

Various dosages of VectoLex CG can provide various degrees of control depending on the site and prevailing environmental conditions. In the real-world situation, extent of control will vary because of these uncontrollable factors. It is evident, nevertheless, that dosages in the range 0.1–2 g/m^2 can provide effective control. During the rainy season, lower dosages will be the method of choice because no long-lasting control of larvae will be expected. In the dry season when the habitat is more stable, higher dosages for lasting control (for a month or longer) should be employed.

The water dispersible granules (WDG) tested in all 4 sites yielded variable results that were influenced by dosage, characteristics of the sites, and precipitation. Two WDG treatments (0.25 g/m^2) at Thanausi produced excellent control for 7–14 days (Fig. 2). At Raevadee site, the 1st WDG treatment (0.1 g/m^2) yielded excellent control for 28 days, whereas the 2nd treatment at the same rate gave good control for 7 days only (Fig. 4). At 15 days posttreatment, immatures increased because of flooding from a canal. Even low dosages of the WDG can provide control for 1 month or so where there is little or no precipitation.

It should be pointed out that heavy rains, flooding, and flushing of the treated sites reduce longevity of control. It should also be noted that some of the sites, such as Wat Lannaboon and Wat Tinnakornnimit, were small and were receiving water from untreated areas upstream. It is possible that immatures from the untreated areas encroached onto the treated plots.

It is also important to note that in using *B. sphaericus* one should not attempt to get 100% control because the existence of larvae aids in recycling: the larvae seem to serve as manufacturers of *B. sphaericus* spores. Once the larvae are completely annihilated, there will be little or no propagation of this bacterial agent, and resurgence of immature mosquitoes will follow. It is therefore advisable to employ moderate dosages rather than high dosages of *B. sphaericus* formulations and achieve moderate to high levels (80–95%) of control rather than to eliminate the total population. But it is equally important to note that higher dosages are apt to give longer control than lower dosages under certain conditions.

ACKNOWLEDGMENTS

We received a great deal of assistance in the conduct of this research from the Division of Medical Entomology, Department of Medical Sciences, National Institutes of Health, Ministry of Public Health of Thailand. Thanks are due to Buncha Tintanon of that Division for ably assisting us in the treatments and sampling of the test sites. We also thank Tianyun Su of the Department of Entomology, University of California, Riverside, for assisting in the preparation of the figures.

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